HISTOLOGICAL STUDY OF IMMATURE INTERSPECIFIC EMBRYO ABORTION BETWEEN *Phaseolus vulgaris* L. And *P. polyanthus* GREENM.

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Introduction

More than 200 pathogens have been reported attacking common bean, some of them causing considerable economic losses (Graham *et al.* 1997, Singh 1999). Good sources of resistance have been found mainly in *Phaseolus coccineus* L. and *P. polyanthus* Greenm. (Baudoin *et al.*, 1992). Some interspecific crosses have been attempted by Lecomte (1997) and Geerts (2001) between *P. polyanthus* (♀) and *P. vulgaris*. Although fertilized ovules were obtained, up to 60 % of globular embryos failed within three to five days to develop due to undefined incompatibility barriers between embryo and mother plant. The aim of this study is to elucidate causes of embryo abortion in reciprocal crosses between *P. vulgaris* and *P. polyanthus*.

Material and Methods

A wild (G 21245) and a cultivated (NI 637) genotype of *P. vulgaris* (PV) and two cultivated genotypes (NI 1015 and G 35348) of *P. polyanthus* (PP) were used. Accessions were selected on the basis of their good ability to flower in growth chamber conditions. Different combinations were made using either PV or PP as female partner. Since all pods obtained by crossing PP (♀) x PV aborted between 5 to 7 days after pollination (DAP), seeds were collected every day, from auto-pollinated flowers and flowers pollinated by either PV or PP. Histological observations were made from these seeds. Methacrylate resin sections, 2 µm thick, of 2 to 6 day-old hybrid seeds were used to examine the stage of embryo development and the state of seed tissues. For each cross, five pods of maternal genotypes (PP or PV), containing three or seven seeds respectively, were examined. These observations are aimed to determine the main causes of abortion and the developmental stages at which interspecific embryos should be rescued.

Results and Discussion

In the comparative histological study, we only considered hybrid seeds, if pollen had germinated on the stigmatic surface and pollen tube residues were observed in sections of the micropylar canal. Number of hybrid seeds observed per cross is summarized in **Table 1**.

Table 1. Numbers of hybrid seeds obtained between *Phaseolus vulgaris* (PV) and *P. polyanthus* (PP) and reciprocal crosses.

Female	***Male***			
	G 21245 (PV)	NI 637 (PV)	NI 1015 (PP)	Total
G 21245 (PV)			123	123
NI 1015 (PP)	46	11		57
G35348 (PP)	37	16		53
Total	83	27	123	233

Embryos aborted at different developmental stages depending on the genotypes used. In more than 20 % of the seeds obtained by using PV (G 21245) as a female, a two-celled embryo could be obtained while less than 10 % of hybrid embryos reached this stage in the reciprocal

crosses PP (♀) x PV (Geerts *et al.*, 2002). When using PV as a female, the first division was initiated 3 DAP and embryo developed to an early globular stage within 6 days in 50 % of the cases. Mature hybrid seeds were obtained. In contrast, when using PP as a female, first division was initiated between 4 to 5 DAP and only 4 embryos out of 107 showed more than two cells 6 DAP. Most of them (74.1 %) did not divide and remained unicellular. All seeds aborted between 6 to 7 DAP.

Differences between early embryo abortion in reciprocal crosses are mainly related to the endosperm development. While a rapid division of primary endosperm nucleus (PEN) is observed in PV (♀) x PP seeds, allowing the further development of the embryo which is initiated 2 to 3 DAP, PEN stay uninucleated in PP (♀) x PV seeds during the first four DAP, limiting nutrient exchange between maternal tissue and zygote. Moreover, our results showed that zygotes of PP (♀) x PV seeds were still able to divide 5 DAP when PEN had divided at least once. This suggests that embryo abortion in PP (♀) x PV seeds could be related to a decrease in nutrient exchange at the beginning of its development, increasing the time at which first division can occur rather than incompatibilities between hybrid embryo and endosperm. This hypothesis is supported by the observations of Lecomte *et al.* (1998) describing wall thickening of the endothelial cells in PP seeds that are tangential while they are radial in PV seeds. Histological differences between maternal tissues in reciprocal crosses could thus be a key factor in the abortion processes.

Later in the hybrid embryo development, the proliferation of the endothelium was clearly described as the main factor of embryo abortion. Differences in the developmental rate of this endothelium proliferation between reciprocal crosses could be attributed to genetic factors or to the rate of endosperm development. In PP (♀) x PV seeds proliferation could be limited to one to five cells in thickness due to the poor endosperm development, while in PV (♀) x PP seeds the development of multinucleated endosperm could lead to a greater endothelial cell proliferation and subsequent later embryo abortion.

We also observed hypertrophy of vascular elements at the chalazal end. Hypertrophy was mainly located at hypostase level. This direct disruption of nutrient transfer was observed in all crosses without significant differences between them. Hypertrophy of vascular elements was observed 3 to 4 DAP and not later (Geerts *et al.*, 2002).

The importance of the abnormalities observed during embryo development depended to a great extent on the compatibility between the genotypes crossed. Results also suggest that the appropriate time for rescue of PP (♀) x PV embryos is at the early globular stage. A pod culture technique was described by Geerts (2001) and Toussaint *et al.* (2002) allowing the development of two-five day-old PV and PP embryos. This technique is now applied to hybrid pods.

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